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***Chloridium terricola* sp. nov. from China**

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ABSTRACT—A new species, *Chloridium terricola*, was isolated from soil in Sichuan province, China. Morphologically, it is characterized by the production of pale brown conidia and a conidiophore bearing a single apical whorl of ampulliform or lageniform phialides. Large subunit (LSU) rDNA sequence analyses support *C. terricola* as congeneric with other *Chloridium* species.

KEY WORDS—hyphomycetes, ITS, phylogenetic, soil fungi, systematics

Introduction

For competing sexually vs. asexually typified generic names in *Sordariomycetes* (except *Diaporthales*, *Hypocreales*, and *Magnaporthales*), Réblová et al. (2016) have recommended the name *Chloridium* Link 1809 based on its priority and widespread use, proposing *Gonytrichum* Nees & T. Nees 1818 and *Melanopsammella* Höhn. 1920 as generic synonyms. In 2005, four hyphomycete isolates morphologically similar to *Chloridium* were obtained from soil in the west region of Sichuan province, China. Their taxonomy was further evaluated through close microscopical examination and large subunit

(LSU) rDNA sequence analyses. These morphological and phylogenetic analyses revealed that the four isolates represented a unique undescribed *Chloridium* species, for which we propose the name *Chloridium terricola*.

Materials & methods

Fungal strains & morphology

Soil samples were collected in Sichuan Province, China, and transported to the laboratory in sterilized, zip lock polyethylene bags. All isolates are maintained in the Plant Pathology Herbarium, Guizhou University, Guiyang, China (HGUP). Mycelium grown from the soil samples was transferred to fresh 1.5% potato dextrose agar (PDA) medium plates. Cultural characteristics and morphology were determined on PDA and incubated for 7 d at 25°C under a 12 h/12 h light and darkness cycle, to promote sporulation. Water solution of 60% (v/v) lactic acid without a color dye was used as the mounting medium. Dimensions are based on 50 mature conidia and 30 conidiophores as measured at 100× magnification under a Nikon 90i microscope.

DNA amplification & sequencing

Extracted DNA was amplified via PCR using primers ITS4 and ITS5 for the ITS region and primers LROR and LR5 for the LSU region (Vilgalys & Hester, 1990; White et al. 1990). Additional sequences were obtained from GenBank. After the sequences were aligned using Clustal X 1.81 (Thompson et al. 1997), the alignments were refined manually. Our DNA sequences were deposited in GenBank.

Phylogenetic analyses

The sequence data were subjected to parsimony analysis using the heuristic search option of PAUP* version 4.0b10 (Swofford 2002). MP (maximum parsimony) trees were inferred using heuristic search option with tree bisection reconnection (TBR) branch swapping and 1000 random sequence additions with maxtrees at 5000, branches of zero length collapsed, and all parsimonious trees saved. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI), and rescaled consistence index (RC). Bootstrap analyses (Hillis & Bull 1993) were conducted with 1000 replications.

Taxonomy

Our morphological and molecular phylogenetic analyses support recognition of our four soil isolates as one phenotypically and phylogenetically distinct species.

Chloridium terricola Yong Wang bis, Jie & K.D. Hyde, *sp. nov.*

FIG. 1

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Differs from *Chloridium gonytrichii* by its single whorl of phialides at the conidiophore apex and by its light brown conidia.

TYPE: China, Sichuan Province, Jiuzhaigou, isolated from soil, August 2005, Yu-Lan Jiang (Holotype, HGUPd4519 [dried culture]; ex-type culture, HGUP4519; GenBank KM434144, KT893302).

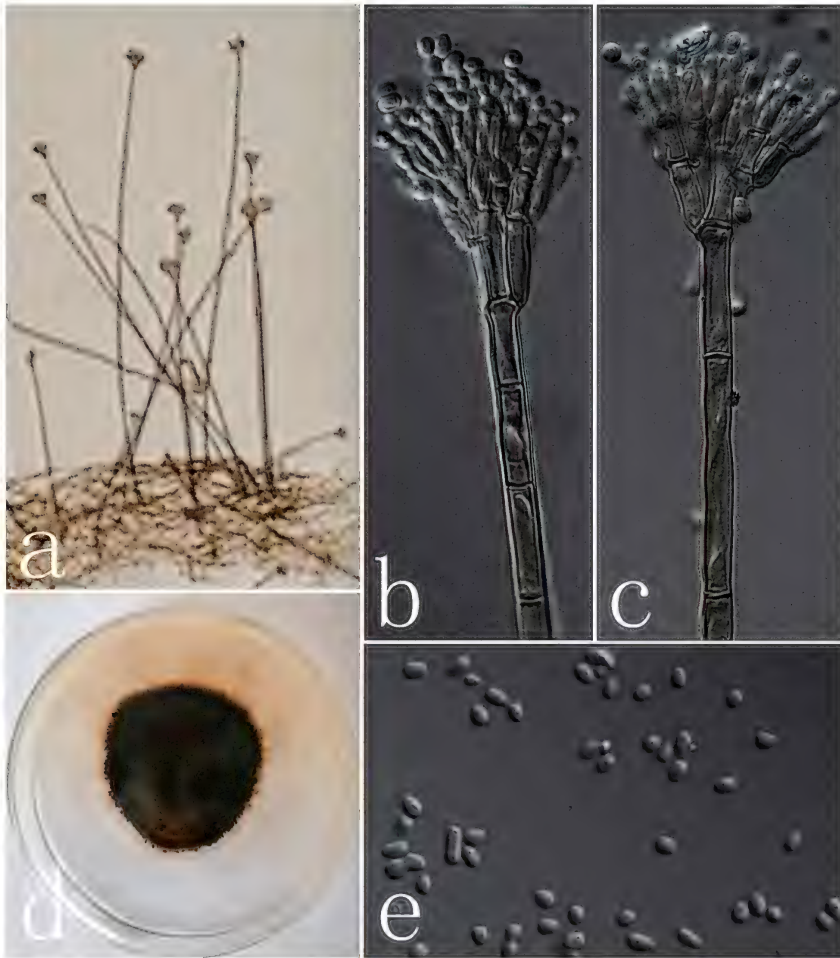


FIG. 1 (color). *Chloridium terricola* (HGUPd4519). a: Conidiophores arising from PDA. b-c: Conidiogenous cells, conidia, and conidiophores. d: Colony on PDA at 7d. e: Conidia. Scale bars: c-d = 20 μ m; e = 10 μ m.

ETYMOLOGY: Latin, *terricola* meaning living on the soil.

COLONIES effuse, velvety, reverse dark brown to black. Mycelium partly immersed, partly superficial, composed of branched, hyaline, septate, smooth, 3.5–5 μ m wide hypha. CONIDIOPHORES mononematous, erect arising from the hypha, hyaline to pale brown, smooth. CONIDIOGENOUS CELLS borne directly on the hypha or in divergent forks on the conidiophores, ampulliform

or lageniform, pale brown, smooth, 7–15 × 2.5–4 µm. CONIDIA obovoid or ellipsoid, truncate at the base, pale brown, smooth, 2–3 × 2–2.5 µm. Sexual stage not observed.

ADDITIONAL SPECIMENS EXAMINED: CHINA, SICHUAN PROVINCE: Panzhihua city, isolated from soil, August 2005, Yu-Lan Jiang (HGUP4509; GenBank KM434143, KT893301); Chengdu city, isolated from rice soil, August 2005, Yu-Lan Jiang (HGUP4536; GenBank KM434146, KT893304); Leshan city, isolated from soil, August 2005, Yu-Lan Jiang (HGUP4521; GenBank KM434145, KT893303).

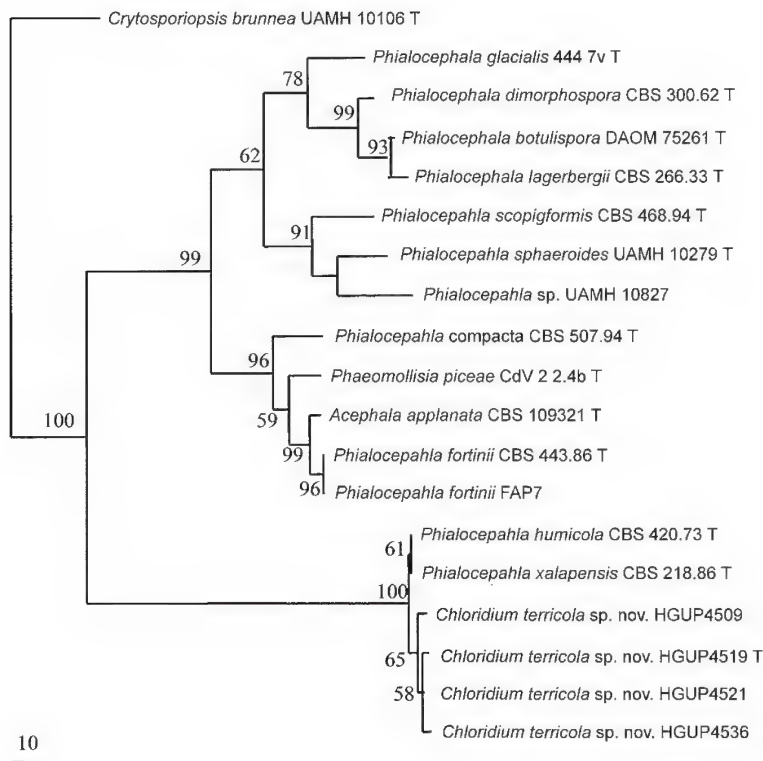
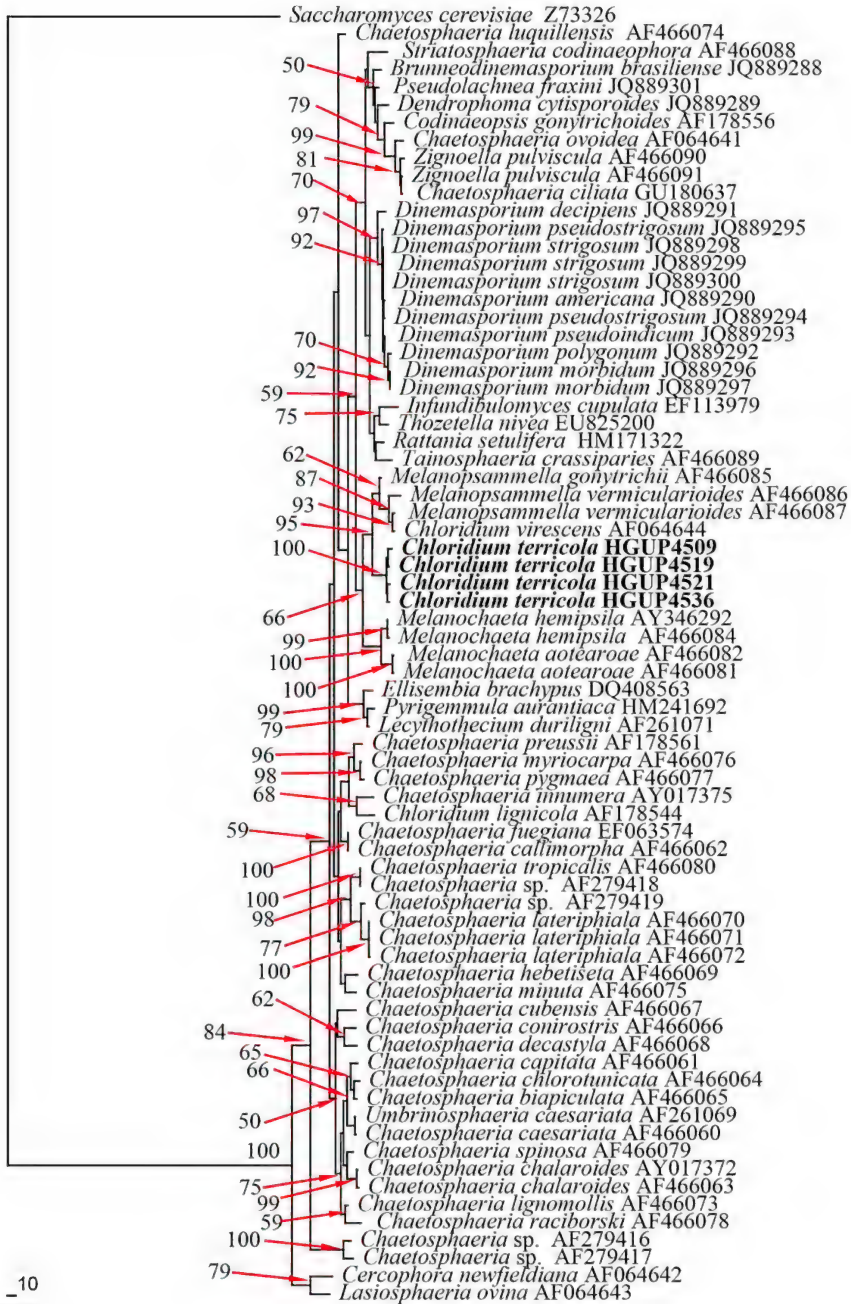


FIG. 2 (above). Topology showing the single most parsimonious tree, inferred from ITS sequence analysis. Bootstrap values less than 50% are not shown. The tree is rooted with *Cryptosporiopsis brunnea* (UAMH 10106). Type specimens are labeled with “T”.

FIG. 3 (right). Topology showing the single most parsimonious tree, inferred from LSU sequence analysis. *Chloridium terricola* (in bold) clusters with *C. gonytrichii* (as *Melanopsammella*) and *C. viridescens* (shown also as *Melanopsammella vermicularioides*). Bootstrap values less than 50% are not shown. The tree is rooted with *Saccharomyces cerevisiae*.



Phylogenetic analysis

An NCBI-BLAST search of ITS sequences showed a 99% DNA identity between our isolates and *Phialocephala humicola* S.C. Jong & E.E. Davis and *P. xalapensis* Persiani & Maggi. We generated a phylogenetic tree of *Phialocephala* spp. based on ITS sequences (FIG. 2), from which we discovered that our taxon was close to, but not conspecific with, *P. humicola* and *P. xalapensis*.

To determine the taxonomic position of our isolates, we used a more inclusive analysis of the LSU region with *Saccharomyces cerevisiae* as outgroup (Crous et al. 2012) and ingroup sequences derived from Crous et al. (2012) and Fernández et al. (2006). Of the 1049 characters aligned, 297 are constant, 496 variable characters are parsimony-uninformative, and 256 are parsimony-informative. One of 32 most parsimonious trees with 1604 steps (CI = 0.610; HI = 0.390; RI = 0.723) derived from analysis of the LSU sequences is presented in FIG. 3. Our four *Chloridium terricola* sequences formed a branch with a 100% BS support (nearly without phylogenetic divergence). They were placed in *Chloridium* group with 100% BS, and showed a closer relationship with *Chloridium gonytrichii* (F.A. Fernández & Huhndorf) Réblová & Seifert and *C. virescens* (Pers.) W. Gams & Hol.-Jech. with high (95%) BS support.

Discussion

The International Code of Nomenclature for algae, fungi, and plants (McNeill et al. 2012) eliminated Art. 59, which in previous botanical codes permitted dual nomenclature for sexual and asexual stages in pleomorphic fungi. As noted above, Réblová et al. (2016) now accept *Melanopsammella* as a synonym of *Chloridium*. Fernández & Huhndorf (2005), who treated the genera before abandonment of Art. 59, noted that *Melanopsammella* species are easily distinguished by their small, one-septate ascospores that disarticulate into partial spores. Sexual stages are almost identical and separation of species is only possible by noting differences in the asexual stage. Because our isolates lacked a sexual stage, we base our morphological comparison on asexual characters.

The obvious morphological difference between *Chloridium terricola* and the asexual stage of *C. gonytrichii* (as *Melanopsammella* in Fernández & Huhndorf 2005) is the number of whorls of phialides: *C. terricola* has only one whorl of phialides (at the conidiophore apex), whereas *C. gonytrichii* has one apical whorl plus 5–8 additional whorls of phialides in the midsection of each conidiophore. Additionally, conidia are pale brown in *C. terricola* and light green conidia in *C. (as Melanopsammella) gonytrichii* (Fernández & Huhndorf 2005).

In comparison to *Chloridium terricola*, *C. virescens* (= *Melanopsammella vermicularioides*; see FIG. 3) conidia are hyaline, uni-/bi-guttulate and longer ($3.2\text{--}4.0 \times 2\text{--}2.5 \mu\text{m}$; Gams & Holubová-Jechová 1976).

Phylogenetically, LSU sequence analysis clusters our taxon with (but taxonomically independent of) *C. gonytrichii* and *C. virescens*, supporting *Chloridium terricola* as a new taxon.

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